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Action of a Fluoranthene-Utilizing Bacterial Community on Polycyclic Aromatic Hydrocarbon Components of Creosote

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16. ABSTRACT

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Action of a Fluoranthene-Utilizing Bacterial Community on Polycyclic Aromatic Hydrocarbon Components of Creosote

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Cultures enriched by serial transfer through a mineral salts medium containing fluoranthene were used to establish a stable, seven-member bacterial community from a sandy soil highly contaminated with coal tar creosote. This community exhibited an ability to utilize fluoranthene as the sole carbon source for growth, as demonstrated by increases in protein concentration and changes in absorption spectra when grown on fluoranthene in liquid culture. Biotransformation of other polycyclic aromatic hydrocarbons (PAHs) was verified by demonstrating their disappearance from an artificial PAH mixture by capillary gas chromatography. When grown on fluoranthene as the sole carbon source and subsequently exposed to fluoranthene plus 16 additional PAHs typical of those found in creosote, this community transformed all PAHs present in this defined mixture. After 3 days of incubation, 13 of the original 17 PAH components were degraded to levels below the limit of detection (10 ng/liter). Continued incubation resulted in extensive degradation of the remaining four compounds. The ability of this community to utilize a high-molecular-weight PAH as the sole carbon source, in conjunction with its ability to transform a diverse array of PAHs, suggests that it may be of value in the bioremediation of environments contaminated with PAHs, such as those impacted by creosote.

Coal tar creosote has been widely used as a wood preservative for over 150 years, with an annual consumption in 1986 estimated at 454,000 metric tons (32). Though creosote-treated products themselves do not appear to represent a threat to the environment, accidental spillage and improper disposal of creosote at production plants and at wood-preserving facilities have resulted in extensive contamination of soil, surface water, and groundwater aquifers (14, 19). Since creosote contains many toxic compounds and priority pollutants (Fed. Regist. 53:41280), such sites are considered hazardous; hence, remedial action is required.

Recent studies have suggested that biodegradation may represent a clean and efficient means of achieving remediation of such sites (6, 28, 33, 39; M. B. Burton, M. M. Martinson, and K. D. Barr, Biotech USA, 5th Annual Industrial Conference, San Francisco, Calif., 14 to 16 November 1988). Nestler (35) reported that 85% of creosote consists of polycyclic aromatic hydrocarbons (PAHs). Therefore, biodegradation of these constituents would result in the removal of a significant volume of creosote pollutants. Moreover, the destruction of these components would significantly reduce the potential health hazards associated with creosote-contaminated environments (25). Likewise, other environments similarly affected by PAHs (e.g., oil refineries and coal gasification sites) may also be improved significantly by removing the hazards associated with this class of chemical pollutant.

Microorganisms capable of degrading certain creosote PAHs have been described, and mechanisms for PAH biodegradation have been reviewed (4, 10). Microbial degradation of lower-molecular-weight PAHs, such as naphthalene (13, 26) and biphenyl (15, 17), by a variety of bacteria is well established. Biotransformation of tricyclic compounds, such as anthracene (24, 27) and phenanthrene (12, 27), has also been reported.

There appear to be no accounts of the microbial utilization of PAHs containing four or more aromatic rings, despite their degradation in contaminated environments (33, 34). However, several publications have described the cometabolism of such PAHs, including benzo[*a*]anthracene (16, 31), benzo[*a*]pyrene (11, 16, 21), fluoranthene (3, 22), and pyrene (23). Incidental metabolism of various PAHs by the lignolytic fungus *Phanerochaete chrysosporium* grown under defined conditions has also been reported (9).

In situ removal of certain creosote pollutants from contaminated environments by the indigenous microflora has been demonstrated (8, 18, 38). Therefore, biotreatability of creosote- and similarly contaminated sites appears potentially effective. To further evaluate the feasibility of such measures and to enhance the overall potential of biodegradation to remove PAHs from contaminated environments, studies were designed to assess the microbiological factors associated with biodegradation of creosote. Our objectives were (i) to screen a creosote-contaminated soil for microorganisms capable of utilizing high-molecular-weight PAHs as the sole source of carbon and energy for growth and (ii) to determine the action of such organisms on combinations of PAHs by using a defined chemical mixture containing PAHs at concentrations typical of those found in coal tar creosote. This work describes the activity of microorganisms isolated with fluoranthene (Fig. 1).

MATERIALS AND METHODS

Source of creosote-degrading microorganisms. Soil highly contaminated with coal tar creosote was freshly obtained from a creosote waste site in Pensacola, Fla. A former evaporation pond for creosote-contaminated wastewater at this site resulted in the formation of a 2-in. (5.08-cm) layer of tarlike sludge heavily contaminated with more than 50% (wt/wt) methylene chloride-extractable organics (unpublished data). Soil immediately adjacent to this sludge was collected from depths of 4 to 8 in. (10.16 to 20.32 cm) and was used as the source of microorganisms in this study. Detailed reports on the history of creosote use, type and

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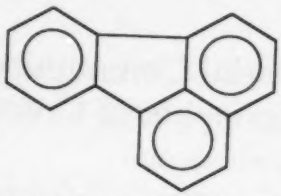

FIG. 1. Chemical structure of fluoranthene.

amount of pollutants present, and the extent of environmental contamination at this site are available (18, 37, 40).

Media. The mineral salts (MS) base used throughout this study consisted of (mg/liter) $(NH_4)_2SO_4$ (1,000), K_2HPO_4 (800), KH_2PO_4 (200), $MgSO_4 \cdot 7H_2O$ (200), $CaCl_2 \cdot 2H_2O$ (100), $FeCl_3 \cdot 6H_2O$ (5), and $(NH_4)_6Mo_4O_{24} \cdot 4H_2O$ (1). The pH was adjusted to 7.0 with 0.1 N HCl, and the medium was sterilized (104 kPa at 121°C for 20 min) prior to the addition of organic substrates.

A MS-plus-fluoranthene (MSF) medium was prepared by adding an excess of fluoranthene (approximately 100 mg/liter) to the MS base. The amount of fluoranthene dissolved by this method is virtually undetectable by measurement of its UV absorption spectrum. This particular medium was sterilized when necessary by autoclaving after the addition of organic substrate. Since this medium was used to supply large amounts of fluoranthene as a sole source of carbon for microbial growth, undissolved fluoranthene crystals were not removed.

To prepare a medium containing a defined mixture of PAHs closely related to the PAH composition of creosote, the appropriate amount of each compound (Table 1) was added to a sterile flask and dissolved in 5.0 ml of methylene chloride to effect sterilization. PAHs (Sigma Chemical Co.) used were of the highest purity available (>98%). Methylene chloride was removed under a stream of dry nitrogen sterilized by passage through a 0.25-μm-pore-size filter, and the PAHs were dissolved by mixing with a magnetic stir bar in an appropriate amount of sterile MS medium. Tween 80 (Sigma Chemical Co.) was added at 200 mg/liter to achieve the aqueous PAH concentrations reported in Table 1. After being mixed for 6 h at room temperature, the medium, designated MSP, was filtered through a layer of sterile glass wool to remove undissolved PAHs. The medium was stored at 4°C in sterile, 1.0-liter Wheaton bottles fitted with Teflon-lined screw caps. The concentration of each compound was determined by capillary gas chromatography of extracted samples as described below.

TABLE 1. Composition of a defined PAH mixture and its relationship to predominant PAHs found in coal tar creosote

Peak no.[a]	Compound	Aqueous solubility[b] (25°C) (mg/liter)	PAH concn in:	
			Defined PAH mixture[c] (mg/liter)	Coal tar creosote[d] (% of total PAHs [range])
1	Naphthalene	31.7	17.1	3.0–15.8
2	2-Methylnaphthalene	25.4	17.1	2.1–14.2
3	1-Methylnaphthalene	28.5	16.0	2.1–14.2
4	Biphenyl	7.5	5.8	2.3–2.8
5	2,6-Dimethylnaphthalene	2.0	2.1	2.0–2.3
6	2,3-Dimethylnaphthalene	3.0	1.9	2.0–2.4
7	Acenaphthene	3.9	3.8	4.1–9.0
8	Fluorene	2.0	3.9	9.6–10.0
9	Phenanthrene	1.3	7.0	4.6–21.0
10	Anthracene	0.07	2.7	1.5–2.0
11	2-Methylanthracene	0.04	0.2	0.5–2.6
12	Anthraquinone		0.9	0.1–1.0
13	Fluoranthene	0.26	8.7	6.8–10.4
14	Pyrene	0.14	2.3	2.2–8.5
15	2,3-Benzo[*b*]fluorene	0.002	0.4	2.0–4.6
16	Chrysene	0.002	0.3	2.8–3.0
17	Benzo[*a*]pyrene	0.003	1.2	0.1–1.0

[a] Order of elution through capillary column SBP-5 (Supelco).
[b] From Baker et al. (2) and Mackay and Shiu (30).
[c] Increased solubility in the presence 200 mg of Tween 80 per liter. The total PAH concentration was 91.4 mg/liter.
[d] Ranges based on analyses by Andersson et al. (1), Becker (5), Borowitzky and Schomburg (7), Lorenz and Gjovik (29), Nestler (35), and Novotny et al. (36).

Fluoranthene enrichment cultures. Fifty milliliters of MSF medium was transferred to a 250-ml screw-cap Erlenmeyer flask and inoculated with 1.0 g (fresh weight) of creosote-contaminated soil passed through a 50-mesh sieve. Flasks were shaken (175 cycles/min) in the dark at 28 ± 1°C. After 5 days of incubation, a 5.0-ml sample was diluted 1:10 (vol/vol) with fresh MSF medium and incubated under the same conditions for 3 days. Subsequent samples were diluted 1:50 with the same medium every 3 days. Following several such transfers, disappearance of undissolved fluoranthene crystals was visually apparent. Enriched cultures apparently containing fluoranthene-utilizing microorganisms were maintained by diluting them 1:50 into fresh MSF medium every 14 days.

Partial characterization of fluoranthene-utilizing community. Numerous samples from fluoranthene enrichment cultures were streaked for isolation on nutrient agar (Difco Laboratories, Detroit, Mich.) amended with 0.5% glucose (NAG agar). After 5 to 14 days of incubation at 28°C, colonies representative of each of the different morphological types were removed and single colonies were repeatedly purified on NAG agar. Eventually, seven morphologically distinct bacteria were isolated in pure culture and were designated FAE1, FAE2, FAE3, FAE4, FAE5, FAE5b, and FAE6.

To ensure that all organisms necessary for fluoranthene utilization had been isolated, MSF medium was inoculated with all seven isolates to reconstitute the community and fluoranthene degradation was assessed. Community as used here is not meant to imply that all seven microorganisms play an obligatory role in PAH degradation in the sense that the term consortium would imply. The community was reconstituted by removing single colonies of each organism from NAG plates and suspending them in sterile MS medium to uniform density (A_{600} = 0.30). Fifty milliliters of MSF medium was inoculated with 0.2 ml of each suspension and incubated at 28°C with aeration (175 cycles/min). Fluoranthene utilization was qualitatively assessed by recording visually apparent increases in microbial biomass, changes in medium coloration, and disappearance of fluoranthene crystals.

Primary utilization of fluoranthene. A sterile 250-ml Erlenmeyer flask fitted with a Teflon-lined screw cap containing 50.0 ml of MSF medium was inoculated with 100 μl of a cell suspension (A_{600} = 0.29) of the reconstituted seven-member community grown in MSF medium. Growth in MS without fluoranthene was also assessed. A killed-cell control was inoculated with 100 μl of the same cell suspension which had been autoclaved. Primary utilization of fluoranthene was demonstrated by monitoring increases in protein concentra-

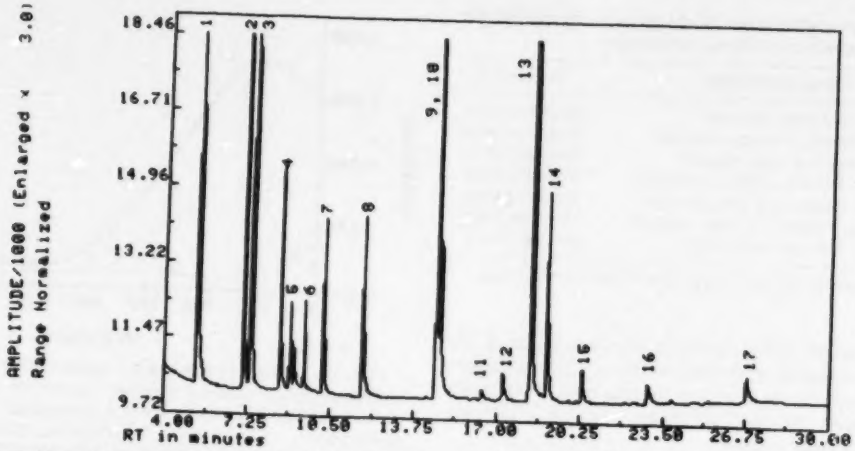

FIG. 2. Gas chromatographic analysis of 17 PAHs extracted from MSP medium. Peak numbers correspond to compounds listed in Table 1. Reference compound 1,4-naphthoquinone is between peaks 5 and 6. RT, Retention time.

tion, the amount of fluoranthene in aqueous solution, and changes in the absorption spectra in the near-UV range, indicative of the formation of fluoranthene metabolites.

At 12-h intervals over 72 h, duplicate 1.0-ml samples were removed, cells were concentrated by centrifugation (12,000 × *g* for 10 min) and lysed in 100 μl of 0.1 N NaOH at 70°C, and the protein concentration was determined by using the enhanced protocol for the BCA protein assay (Pierce Chemical Co., Rockford, Ill.). Additionally, two separate 1.0-ml samples were filtered through 0.2-μm Teflon-coated nylon membranes (Gelman Sciences, Ann Arbor, Mich.). One filtered sample was used to monitor changes in absorbance of the filtrate between 300 and 500 nm with a Hewlett-Packard model 8452A diode array spectrophotometer. High-performance liquid chromatographic analysis of the remaining filtered sample was performed as described below to determine the aqueous fluoranthene concentration and to detect the presence of fluoranthene metabolites.

Action of bacterial cells toward other PAHs. Eight clean, sterile 50.0-ml test tubes, fitted with Teflon-lined screw caps, containing 5.0 ml of MSP medium were inoculated with 100 μl of a cell suspension (A_{600} = 0.32) of the reconstituted community grown on fluoranthene. Killed-cell controls were generated by adding 500 μl of a 37% Formalin solution. Uninoculated controls were also included. Another set of tubes was inoculated with 100 μl of a cell suspension (A_{600} = 0.31) of *Pseudomonas putida* PpG7 (NAH7), ATCC 17485 (a gift from I. C. Gunsalus, University of Illinois, Urbana). After 3, 5, 8, and 14 days of incubation in the dark (28°C, 200 cycles/min), duplicate tubes of each treatment were removed and the contents were extracted for determination of PAHs.

For each time of sampling, the entire contents (5.0 ml) of a given tube were transferred to a clean, methylene chloride-rinsed, 15-ml glass conical extraction tube fitted with a Teflon-lined screw cap. To this was added 2.0 ml of methylene chloride used to rinse the reaction tube. The tubes were shaken for 1.0 min to extract unmetabolized PAHs into the organic phase. After centrifugation (2,500 × *g* for 5 min) the methylene chloride phase was transferred with a methylene chloride-rinsed, 1.0-ml glass syringe fitted with a blunt-end needle to a clean, methylene chloride-rinsed, 10.0-ml Kuderna-Danish concentration tube. The extraction procedure was repeated twice with 0.5 ml of methylene chloride. The pooled methylene chloride extract (approximately 3.0 ml) was passed over a 0.5-cm layer of anhydrous sodium sulfate held in a glass funnel, and the volume was reduced to <1.0 ml under a stream of dry nitrogen. The final volume of methylene chloride was adjusted to 1.0 ml, and each extract received 10 μl of a 1,4-naphthoquinone solution (10 mg/ml of methylene chloride) as a marker before being transferred to a gas chromatography vial and crimp sealed.

Analytical methods. Gas chromatographic analyses of methylene chloride extracts of MSP medium (Fig. 2) and of individual PAH standards were performed on a Hewlett-Packard model 5710A gas chromatograph equipped with a flame ionization detector. Hydrogen was used as the carrier gas (0.5 ml/min), while air (240 ml/min) and hydrogen (30 ml/min) were supplied for the flame ionization detector. PAHs in replicate 1.0-μl injections were separated on an SPB-5 (Supelco, Bellefonte, Pa.) capillary column (15.0 m by 0.32 mm [inside diameter] with a 0.25-μm coating phase). The oven temperature was programmed at 80°C for 2 min followed by a linear increase of 8°C/min to 280°C, at which it was held for 4 min. Injector and detector temperatures were maintained at 270°C. The percent recovery of each PAH by methylene chloride extraction was calculated by comparing its peak area with that of a standard of each compound.

High-performance liquid chromatography was performed on a Hewlett-Packard model 1090M HPLC equipped with a diode array detector and an octadecylsilane-Hypersil (microbore, C-18; 100 mm by 2.1 mm [inside diameter]) column (Hewlett-Packard Co., Palo Alto, Calif.). Solvents A (90% 50 mM KH_2PO_4 [pH 3.5]) and B (10% acetonitrile) were used (flow rate, 0.5 ml/min) to separate replicate 10.0-μl injections. The initial concentration of solvent B was 10% and increased at 3%/min to 82% at the end of the run. Sample wavelengths used to determine aqueous fluoranthene concentrations were 204, 234, and 460 nm, and the peak area (fluoranthene retention time, 21 min) was compared with that of a 1.0 mM fluoranthene standard.

RESULTS

Enrichment cultures obtained through serial transfer in a MS medium containing fluoranthene resulted in a microbial community capable of utilizing fluoranthene as the sole source of carbon and energy for growth. Fluoranthene utilization in the enriched cultures was evidenced by visual

TABLE 2. Partial characterization of the bacteria composing the fluoranthene-utilizing community

Strain	Colony morphology[a]	Gram reaction
FAE1	White, 1–2 mm, mucoid	Negative rods
FAE2	Light brown, 3–4 mm, mucoid	Negative cocci
FAE3	Colorless, 1–2 mm, mucoid	Negative cocci
FAE4	White, 3–4 mm, slime producing	Negative rods
FAE5	Bright yellow, 1–2 mm, mucoid	Negative rods
FAE5b	Opaque yellow, <1 mm, mucoid	Negative rods
FAE6	White, 4–5 mm, spreading	Positive cocci

[a] Colony morphology after 5 days of incubation at 28°C on NAG agar.

changes in the amount of fluoranthene crystals, color of the medium, and increases in microbial biomass. Color changes occurred from colorless to bright orange to bright yellow to a light brown, with the last persisting after fluoranthene crystals were no longer visible.

When fluoranthene-enriched cultures were plated on a complex medium (NAG agar) a total of seven morphologically distinct bacteria were isolated (Table 2). These seven colony types appeared in the first plating and every plating thereafter. When MSF medium was inoculated with a reconstituted cell suspension of this community, fluoranthene degradation was again obvious following an initial lag of 5 to 7 days. After this lag period, increases in microbial biomass were apparent, fluoranthene crystals disappeared, and the sequence of color changes was complete within 2 days.

The fluoranthene-utilizing ability of the community was more quantitatively assessed by measuring increases in bacterial protein and monitoring changes in UV absorbance. Cultures growing on fluoranthene showed protein concentrations from 5.5 to 62.7 μg/ml after 60 h of incubation (Fig. 3). No increases in protein were apparent with the killed-cell control or in the absence of fluoranthene. The initial concentration of fluoranthene in aqueous solution was 0.21 μg/ml. Aqueous fluoranthene concentrations remained low but detectable in killed-cell controls, whereas they were below the limit of detection after 12 h of incubation with the fluoranthene-utilizing community (data not shown). Corresponding increases in absorbance between 300 and 500 nm, associated

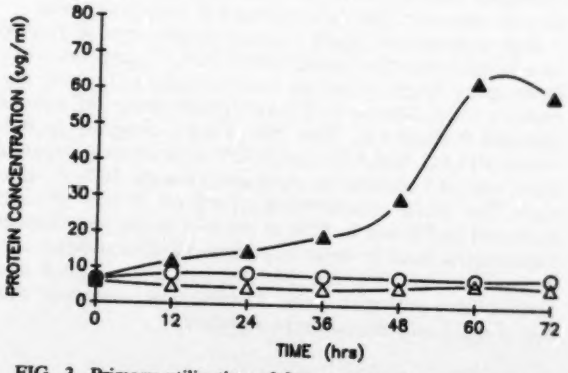

FIG. 3. Primary utilization of fluoranthene by a seven-member bacterial community. Shown are protein determinations assessing primary utilization of fluoranthene in MSF liquid medium inoculated with a seven-member bacterial community (▲) and MSF medium inoculated with killed cells of the community (△). Changes in bacterial protein in the absence of fluoranthene are also presented (○).

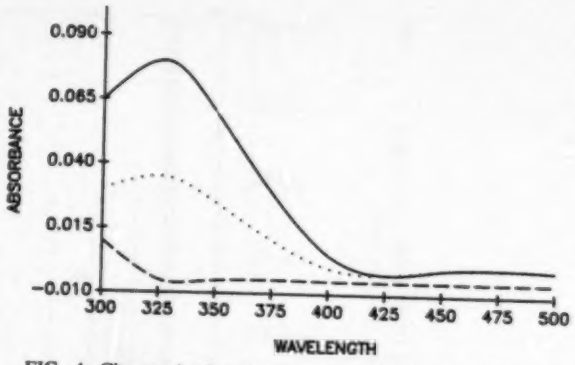

FIG. 4. Changes in absorbance spectra of a bacterial community in fluoranthene-containing medium. Shown are absorbance characteristics of MSF medium freshly inoculated with the bacterial consortium (– – –) and of the same medium after 36 (· · · ·) and 72 (——) h of incubation at 28°C with shaking (175 cycles/min). Data correspond with those presented in Fig. 3.

with change in medium coloration, were observed only with actively growing cells (Fig. 4).

The percent recoveries from MSP medium of 17 PAHs 3 days after inoculation with either the fluoranthene-grown reconstituted community or with *P. putida* PpG7 are given in Table 3. Extraction efficiencies and losses attributable to abiotic processes were accounted for by comparing recovery values for each compound with those obtained from the killed-cell controls. Except for naphthalene (84.3%) and 2,3-dimethylnaphthalene (78.9%), the percent recovery from abiotic controls was greater than 85%.

The ability to detect selective utilization of individual components of the defined mixture was demonstrated with

TABLE 3. Percent recovery of 17 PAHs by a seven-member, fluoranthene-grown bacterial community after incubation for 3 days

Compound	% Recovery[a] of PAHs		
	Fluoranthene-grown community	Killed cells[b]	*P. putida* PpG7
Naphthalene	ND[c]	84.3 ± 2.3[d]	ND
2-Methylnaphthalene	ND	85.7 ± 10.3	ND
1-Methylnaphthalene	ND	85.2 ± 7.3	64.5 ± 3.0
Biphenyl	ND	86.6 ± 13.8	89.7 ± 1.8
2,6-Dimethylnaphthalene	ND	91.4 ± 4.8	97.1 ± 2.9
2,3-Dimethylnaphthalene	ND	78.9 ± 12.7	82.5 ± 2.5
Acenaphthene	ND	96.3 ± 3.7	102.4 ± 0.8
Fluorene	ND	106.2 ± 11.8	117.8 ± 10.7
Phenanthrene	ND	91.2 ± 10.8	119.4 ± 11.4
Anthracene	1.5 ± 1.5	91.5 ± 4.1	100.6 ± 1.6
2-Methylanthracene	ND	100.0 ± 30.0	67.0 ± 2.0
Anthraquinone	6.5 ± 6.5	88.9 ± 17.8	85.6 ± 14.8
Fluoranthene	69.5 ± 13.6	101.2 ± 9.8	102.1 ± 18.8
Pyrene	44.3 ± 8.6	87.0 ± 8.7	108.9 ± 8.1
2,3-Benzo[*b*]fluorene	ND	115.0 ± 15.0	120.0 ± 5.0
Chrysene	ND	106.7 ± 16.3	126.7 ± 7.7
Benzo[*a*]pyrene	ND	115.6 ± 7.7	114.0 ± 14.2

[a] Calculations based on the recovery of each individual chemical from the killed-cell control.

[b] Fluoranthene-grown bacterial cells killed with 10% formaldehyde (37% Formalin solution) at the time of inoculation.

[c] ND, Not detected (<0.01 mg/liter).

[d] Data presented are the means of duplicate samples ± the standard deviations of the means.

TABLE 4. Changes in amounts of four PAHs recoverable from medium inoculated with the fluoranthene-utilizing community at different times

Compound	% Recovery at indicated time (days) after inoculation[a]		
	5	8	14
Anthracene	ND	ND	ND
Anthraquinone	ND	ND	ND
Fluoranthene	52.1 ± 22.3	41.6 ± 8.8	16.8 ± 6.1
Pyrene	43.9 ± 12.6	17.4 ± 7.0	12.0 ± 12.0

[a] Data are the means of duplicate samples ± the standard deviations of the means. ND, Not detected (<0.01 mg/liter).

the medium inoculated with strain PpG7 (Table 3). After 3 days of incubation, only naphthalene and 2-methylnaphthalene were extensively degraded. These data are identical to those obtained after 5, 8, and 14 days of incubation (data not shown).

When the community was grown on fluoranthene and subsequently exposed to fluoranthene plus 16 other PAHs, it degraded all of the PAHs present in the defined mixture after 3 days (Table 3). Thirteen of the original 17 PAHs were degraded to below the limits of detection (10 ng/liter). Additionally, greater than 90% degradation of anthracene and anthraquinone was apparent. The remaining two compounds, fluoranthene and pyrene, were also degraded to some extent. However, fluoranthene recoveries may be misleading because of the carryover of this chemical from the inoculum. Community biomass for inoculation was generated in MSF medium which contained an excess of insoluble fluoranthene (100 mg/liter). It could be calculated that tubes inoculated with the fluoranthene community received an additional 0.38 mg of fluoranthene, resulting in an initial fluoranthene concentration of 83.8 mg/liter in MSF medium. Therefore, after 3 days of incubation, the fluoranthene-induced community had degraded 30% of the total amount of fluoranthene originally present or a total of 0.13 mg of fluoranthene.

With continued incubation, further degradation of the four compounds which were still present at 3 days was observed (Table 4). After 5 days of incubation, anthracene and anthraquinone were no longer detectable. The amounts of fluoranthene and pyrene evident after 5, 8, and 14 days of incubation decreased steadily. No losses of these compounds were discernible with the killed-cell control or in the presence of strain PpG7 (data not shown).

DISCUSSION

When evaluating the potential of bioremediation for treating creosote-contaminated sites, the technology is immediately challenged by the presence of many different, structurally diverse pollutants. For example, Nestler (35) compiled a list of over 162 individual components found in coal tar creosote. Despite this array of chemicals, creosote may be more simply viewed as consisting of three fractions: a PAH fraction representing the majority of the constituents (85%), a phenolic fraction (10%), and a heterocyclic fraction (5%) (35).

Since creosote consists primarily of PAHs, experiments were designed to assess the biodegradability of this fraction. By and large, individual PAHs previously detected in creosote at concentrations >1% were present in the defined mixture used in this study. Though other high-molecular-weight PAHs such as benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and dibenz[*a*,*h*]anthracene have been detected in creosote, for the purposes of this investigation they were omitted from the defined mixture. Use of such a mixture proved advantageous for the isolation of biological systems capable of degrading complex chemical mixtures, as are often found at hazardous waste sites.

Our study showed that a bacterial community isolated from a creosote waste site was capable of utilizing fluoranthene as the sole source of carbon and energy for bacterial growth. To our knowledge, this is the first report of primary utilization of a PAH containing four or more rings. No attempt was made at this stage of our investigation to identify the materials responsible for the color changes seen as fluoranthene utilization occurred. However, color changes of this kind are often associated with the transient accumulation of catechol-like compounds and their *meta*-ring fission products.

After growth on fluoranthene, this community was capable of degrading other PAHs present in the defined mixture. Observed losses of PAHs were probably the result of both primary utilization and cometabolism. Whereas the fluoranthene-grown community was capable of utilizing fluoranthene as the sole carbon source, it is not yet known which other compounds may be similarly degraded and which are cometabolized. It was shown that strain PpG7 was active against both naphthalene and 2-methylnaphthalene but showed little activity against the other PAHs present. Presumably, oxygenation of the remaining PAHs is not readily effected by strain PpG7, but organisms in the fluoranthene-utilizing community apparently possess the enzymes with the requisite specificity. Another consideration is the presence of Tween 80. While Tween 80 represents an additional potential source of carbon for growth, it is not essential for PAH degradation but serves to enhance biodegradation of high-molecular-weight, hydrophobic chemicals by increasing their aqueous solubilities, thereby allowing monitoring of their disappearance (20).

The roles of the seven different microorganisms in the fluoranthene-utilizing community remain to be established. It is possible that some members supply the requisite oxygenases and other degradative enzymes while others supply ancillary functions such as surfactant production or provision of growth factors. Until it can be shown which of these organisms is essential for the utilization of fluoranthene, it is more loosely described as a community rather than a consortium. Research is in progress to resolve these questions.

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